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PATENT
1817-0105P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	SHVETS, et al.	Conf.:	
Appl. No.:	09/750,348	Group:	Unknown
Filed:	December 29, 2000	Examiner:	Unknown
For:	BIOLOGICAL ASSAYS		

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

March 22, 2001

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please replace paragraph 1 on page 3 of the specification with the following paragraph:

The most commonly used cell transmigration assay is a modified "Boyden chamber" assay such as described in US Patent Specification No. 5578492 (Fedun et al). This involves assessing thecrossing of a quantity of cells through a

microporous membrane under the influence of a chemoattractant, recombinant or cell-derived. Here the diameter of the micropores are less than the diameter of the cells under investigation, such that the cells must deform themselves in order to squeeze through the pores thereby constructing an analogy to the transendothelial migration of cells in psysiological circumstances. Once the cells are deposited onto the membrane, the chamber can be incubated for intervals over time at a suitable temperature, usually 37°. Following this, the bottom chamber or opposite side of the top chamber may be analysed for cells that have squeezed through the microporous membrane.

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REMARKS

Paragraph 1 on page 3 of the specification has been amended to correct US Specification No. 5,578,192 to 4,478,492. A copy of the marked up version of this paragraph 1 on page 3 is attached.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Joseph A. Kolasch (Reg. 22,463) at the telephone number of the undersigned below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Joseph A. Kolasch, #22,463

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

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switch to the motile phenotype. Motility assays are assessed by estimating the ratio of cells undergoing cytoskeletal rearrangements and the formation of uropods (extension of the trailing tail). One of the major disadvantages of this and the previous adhesion assays is the geometrical design (microscope slides and multiple well chambers), which does not at all resemble the in vivo situation.

The most commonly used cell transmigration assay is a modified "Boyden chamber" assay such as described in US Patent Specification No. 5578492 (Fedun et al). This involves assessing the crossing of a quantity of cells through a microporous membrane under the influence of a chemoattractant, recombinant or cell-derived. Here the diameter of the micropores are less than the diameter of the cells under investigation, such that the cells must deform themselves in order to squeeze through the pores thereby constructing an analogy to the transendothelial migration of cells in physiological circumstances. Once cells are deposited onto the membrane, the chamber can be incubated for intervals over time at a suitable temperature, usually 37°. Following this, the bottom chamber or opposite side of the top chamber may be analysed for cells that have squeezed through the microporous membrane.

US Patent Specification Nos. 4912057 (Guirguis et al), 5284753 (Goodwin et al), 5302515 (Goodwin et al), 5514555 (Springer et al) and 5601997 (Tchao) are typical examples of these assays. The main disadvantage of the assays described in those specifications is that the biological process of transmigration through the micropores is difficult to observe due to the geometrical configuration of the apparatus involved. The lens of the optically inverted microscope must be able to focus through the lower chamber and the microporous membrane. This obviously leads to difficulties due to optical aberrations. In effect, the study of the cells morphology changes while transmigrating across the membrane and their subsequent cytoskeletal changes reverting to their former state is a process which is difficult to monitor and record due to limitations with current techniques. In addition, although it is possible to alter such an experiments parameters following the initiation of the experiment, such as the introduction of a second chemoattractant, recombinant or cell-derived, at some specified time after commencing the experiment, it is not possible to distinguish separate effects from each said chemoattractant.